

Exhibit A

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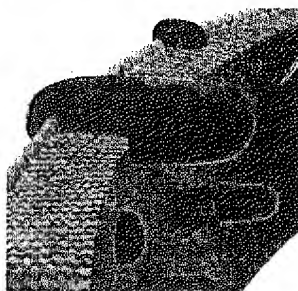
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11

Lipids and Membranes

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elles, such as nuclei, mitochondria, chloroplasts, the endoplasmic reticulum, and the Golgi apparatus (Fig. 1-5), are likewise membrane bounded.

Biological membranes are organized assemblies of lipids and proteins with small amounts of carbohydrate. Yet they are not impermeable barriers to the passage of materials. Rather, they regulate the composition of the intracellular medium by controlling the flow of nutrients, waste products, ions, etc., into and out of the cell. They do this through membrane-embedded "pumps" and "gates" that transport specific substances against an electrochemical gradient or permit their passage with such a gradient (Chapter 18).

Many fundamental biochemical processes occur on or in a membranous scaffolding. For example, electron transport and oxidative phosphorylation (Chapter 20), processes that oxidize nutrients with the concomitant generation of ATP, are mediated by an organized battery of enzymes that are components of the inner mitochondrial membrane. Likewise, photosynthesis, in which light energy powers the chemical combination of H_2O and CO_2 to form carbohydrates (Chapter 22), occurs in the inner membranes of chloroplasts. The processing of information, such as sensory stimuli or intercellular communications, is generally a membrane-based phenomenon. Thus nerve impulses are mediated by nerve cell membranes (Section 34-4C) and the presence of certain substances such as hormones and nutrients is detected by specific membrane-bound receptors (Section 34-4B).

In this chapter, we examine the compositions and structures of biological membranes and related substances. Specific membrane-based biochemical processes, such as those mentioned above, are dealt with in the later chapters.

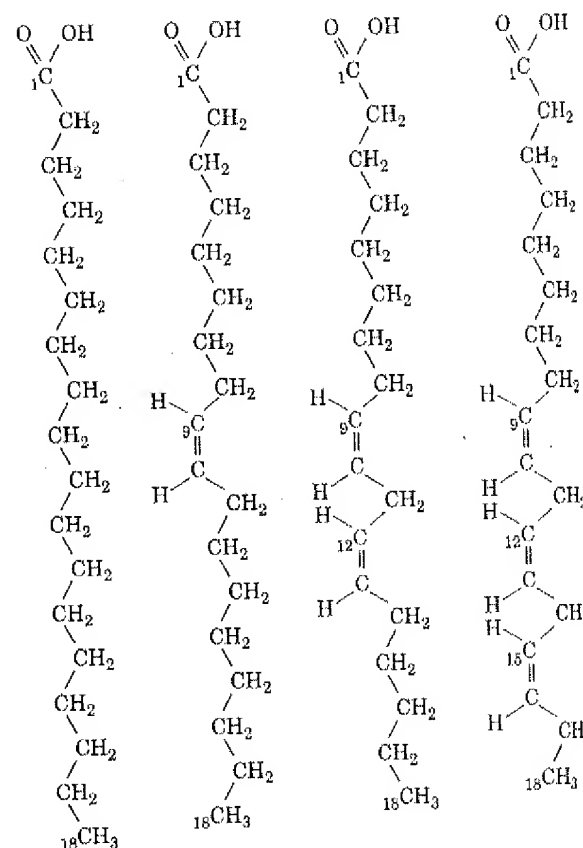
Membranes function to organize biological processes by compartmentalizing them. Indeed, the cell, the basic unit of life, is essentially defined by its enveloping plasma membrane. Moreover, in eukaryotes, many subcellular organ-

1. LIPID CLASSIFICATION

Lipids (Greek: *lipos*, fat) are substances of biological origin that are soluble in organic solvents such as chloroform and methanol but are only sparingly soluble, if at all, in water. Hence, they are easily separated from other biological materials by extraction into organic solvents and may be further fractionated by such techniques as adsorption chromatography, thin layer chromatography, and reverse-phase chromatography (Section 5-3E). Fats, oils, certain vitamins and hormones, and most nonprotein membrane components are lipids. In this section, we discuss the structures and physical properties of the major classes of lipids.

A. Fatty Acids

Fatty acids are carboxylic acids with long-chain hydrocarbon side groups (Fig. 11-1). They are rarely free in nature but, rather, occur in esterified form as the major components of the various lipids described in this chapter. The more common biological fatty acids are listed in Table 11-1. In higher plants and animals, the predominant fatty acid residues are those of the C_{16} and C_{18} species **palmitic**, **oleic**, **linoleic**, and **stearic acids**. Fatty acids with <14 or >20 carbon atoms are uncommon. Most fatty acids have an even number of carbon atoms because they are usually biosynthesized by the concatenation of C_2 units (Section 23-4C). Over half of the fatty acid residues of plant and animal lipids are unsaturated (contain double bonds) and are often



Stearic acid Oleic acid Linoleic acid α-Linolenic acid

FIGURE 11-1. The structural formulas of some C_{18} fatty acids. The double bonds all have the *cis* configuration.

TABLE 11-1. THE COMMON BIOLOGICAL FATTY ACIDS

Symbol ^a	Common Name	Systematic Name	Structure	mp
Saturated fatty acids				
12:0	Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	
14:0	Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	
16:0	Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	
18:0	Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	
20:0	Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	
22:0	Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	
24:0	Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	
Unsaturated fatty acids (all double bonds are <i>cis</i>)				
16:1	Palmitoleic acid	9-Hexadecenoic acid	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$	
18:1	Oleic acid	9-Octadecenoic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	
18:2	Linoleic acid	9,12-Octadecadienoic acid	$CH_3(CH_2)_4(CH=CHCH_2)_2(CH_2)_6COOH$	
18:3	α-Linolenic acid	9,12,15-Octadecatrienoic acid	$CH_3CH_2(CH=CHCH_2)_3(CH_2)_3COOH$	
18:3	γ-Linolenic acid	6,9,12-Octadecatrienoic acid	$CH_3(CH_2)_4(CH=CHCH_2)_3(CH_2)_3COOH$	
20:4	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$	
20:5	EPA	5,8,11,14,17-Eicosapentanoic acid	$CH_3CH_2(CH=CHCH_2)_5(CH_2)_2COOH$	
24:1	Nervonic acid	15-Tetracosenoic acid	$CH_3(CH_2)_7CH=CH(CH_2)_{15}COOH$	

^a Number of carbon atoms : Number of double bonds.

Source: Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), Chapter 8, Clarendon Press (1956).

polyunsaturated (contain two or more double bonds). Bacterial fatty acids are rarely polyunsaturated but are commonly branched, hydroxylated, or contain cyclopropane rings. Unusual fatty acids also occur as components of the oils and waxes (esters of fatty acids and long-chain alcohols) produced by certain plants.

The Physical Properties of Fatty Acids Vary with Their Degree of Unsaturation

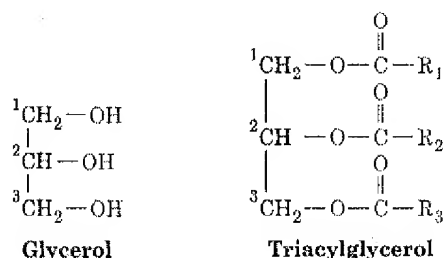
Table 11-1 indicates that the first double bond of an unsaturated fatty acid commonly occurs between its C9 and C10 atoms counting from the carboxyl C atom (a Δ^9 - or 9-double bond). In polyunsaturated fatty acids, the double bonds tend to occur at every third carbon atom towards the methyl terminus of the molecule (such as $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$). Double bonds in polyunsaturated fatty acids are almost never conjugated (as in $-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$). Triple bonds rarely occur in fatty acids or any other compound of biological origin.

Saturated fatty acids are highly flexible molecules that can assume a wide range of conformations because there is relatively free rotation about each of their C—C bonds. Nevertheless, their fully extended conformation is that of minimum energy because this conformation has the least amount of steric interference between neighboring methylene groups. The melting points (mp) of saturated fatty acids, like those of most substances, increase with molecular mass (Table 11-1).

Fatty acid double bonds almost always have the *cis* configuration (Fig. 11-1). This puts a rigid 30° bend in the hydrocarbon chain of unsaturated fatty acids that interferes with their efficient packing to fill space. The consequent reduced van der Waals interactions cause fatty acid melting points to decrease with their degree of unsaturation (Table 11-1). Lipid fluidity likewise increases with the degree of unsaturation of their component fatty acid residues. This phenomenon, as we shall see in Section 11-3B, has important consequences for membrane properties.

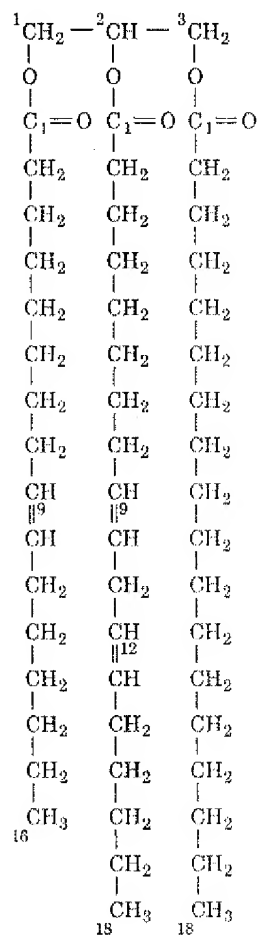
B. Triacylglycerols

The fats and oils that occur in plants and animals consist largely of mixtures of **triacylglycerols** (also referred to as **triglycerides** or **neutral fats**). These nonpolar, water-insoluble substances are fatty acid triesters of **glycerol**:



Triacylglycerols function as energy reservoirs in animals and are therefore their most abundant class of lipids even though they are not components of biological membranes.

Triacylglycerols differ according to the identity and placement of their three fatty acid residues. The so-called **simple triacylglycerols** contain one type of fatty acid residue and are named accordingly. For example, **tristearoylglycerol** or **tristearin** contains three stearic acid residues, whereas **trioleoylglycerol** or **triolein** has three oleic acid residues. The more common **mixed triacylglycerols** contain two or three different types of fatty acid residues and are named according to their placement on the glycerol moiety.



1-Palmitoleoyl-2-linoleoyl-3-stearoyl-glycerol

Fats and oils (which differ only in that fats are solid and oils are liquid at room temperature) are complex mixtures of simple and mixed triacylglycerols whose fatty acid compositions vary with the organism that has produced them. Plant oils are usually richer in unsaturated fatty acid residues than are animal fats, as the lower melting points of oils imply.



FIGURE 11-2. A scanning electron micrograph of adipocytes. Each contains a fat globule that occupies nearly the entire cell. [Fred E. Hossler/Visuals Unlimited.]

Triacylglycerols Function as Energy Reserves

Fats are a highly efficient form in which to store metabolic energy. This is because fats are less oxidized than are carbohydrates or proteins and hence yield significantly more energy on oxidation. Furthermore, fats, being nonpolar substances, are stored in anhydrous form, whereas glycogen, for example, binds about twice its weight of water under physiological conditions. Fats therefore provide about six times the metabolic energy of an equal weight of hydrated glycogen.

In animals, **adipocytes** (fat cells; Fig. 11-2) are specialized for the synthesis and storage of triacylglycerols. Whereas other types of cells have only a few small droplets of fat dispersed in their cytosol, adipocytes may be almost entirely filled with fat globules. **Adipose tissue** is most abundant in a subcutaneous layer and in the abdominal cavity. The fat content of normal humans (21% for men, 26% for women) enables them to survive starvation for 2 to 3 months. In contrast, the body's glycogen supply, which functions as a short-term energy store, can provide for the body's metabolic needs for less than a day. The subcutaneous fat layer also provides thermal insulation, which is particularly important for warm-blooded aquatic animals, such as whales, seals, geese, and penguins, which are routinely exposed to low temperatures.

C. Glycerophospholipids

Glycerophospholipids (or **phosphoglycerides**) are the major lipid components of biological membranes. They consist of **sn-glycerol-3-phosphate** (Fig. 11-3a) esterified at its C1 and C2 positions to fatty acids and at its phosphoryl group to a group, X, to form the class of substances diagrammed in Fig. 11-3b. *Glycerophospholipids are therefore amphiphilic molecules with nonpolar aliphatic "tails" and polar phosphoryl-X "heads."* The simplest glycerophospholipids, in which X = H, are **phosphatidic acids**; they are present only in small amounts in biological membranes. *In the glycerophospholipids that commonly occur in biological membranes, the head groups are derived from polar alcohols* (Table 11-2). Saturated C₁₆ and C₁₈ fatty acids usually occur at the C1 position of glycerophospholipids and the C2 position is often occupied by an unsaturated C₁₆ to C₂₀ fatty acid. Glycerophospholipids are, of course, also named according to the identities of these fatty acid residues (Fig. 11-4). Some glycerophospholipids have common names. For example, phosphatidylcholines are known as **lecithins**; diphosphatidylglycerols, the "double" glycerophospholipids, are known as **cardiolipins** (because they were first isolated from heart muscle).

Plasmalogens are glycerophospholipids in which the C1 substituent to the glycerol moiety is bonded to it via an

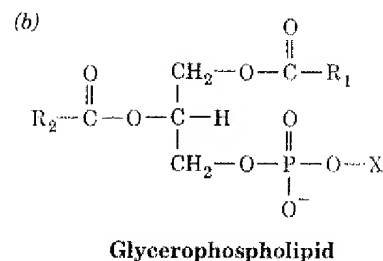
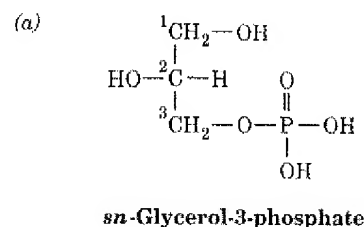
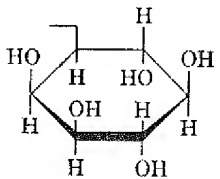
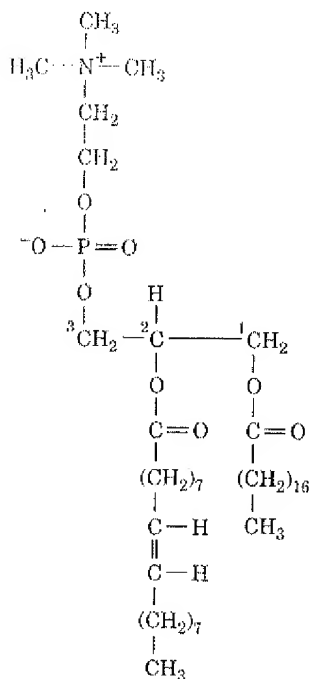


FIGURE 11-3. (a) The compound shown in Fischer projection (Section 4-2B) can be equivalently referred to as L-glycerol-3-phosphate or D-glycerol-1-phosphate. However, using stereospecific numbering (*sn*), which assigns the 1-position to the group occupying the *pro-S* position of a prochiral center (see Section 4-2C for a discussion of prochirality), the compound is unambiguously named *sn*-glycerol-3-phosphate. (b) The general formula of the glycerophospholipids. R₁ and R₂ are long-chain hydrocarbon tails of fatty acids and X is derived from a polar alcohol (see Table 11-2).

TABLE 11-2. THE COMMON CLASSES OF GLYCEROPHOSPHOLIPIDS

$ \begin{array}{c} \text{O} \\ \parallel \\ \text{R}_2-\text{C}-\text{O}-\text{CH}_2-\text{O}-\text{C}-\text{R}_1 \\ \parallel \\ \text{O} \\ \text{CH} \\ \parallel \\ \text{CH}_2-\text{O}-\text{P}-\text{O}-\text{X} \\ \parallel \\ \text{O}^- \end{array} $		
Name of X—OH	Formula of —X	Name of Phospholipid
Water	—H	Phosphatidic acid
Ethanolamine	—CH ₂ CH ₂ NH ₃ ⁺	Phosphatidylethanolamine
Choline	—CH ₂ CH ₂ N(CH ₃) ₃ ⁺	Phosphatidylcholine (lecithin)
Serine	—CH ₂ CH(NH ₃ ⁺)COO ⁻	Phosphatidylserine
myo-Inositol		Phosphatidylinositol
Glycerol	—CH ₂ CH(OH)CH ₂ OH	Phosphatidylglycerol
Phosphatidylglycerol	$ \begin{array}{c} \text{O} \\ \parallel \\ -\text{CH}_2\text{CH}(\text{OH})\text{CH}_2-\text{O}-\text{P}-\text{O}-\text{CH}_2 \\ \parallel \quad \quad \quad \parallel \\ \text{O}^- \quad \quad \quad \text{O} \\ \text{O} \quad \quad \quad \parallel \\ \parallel \quad \quad \quad \text{CH}-\text{O}-\text{C}-\text{R}_4 \\ \text{R}_3-\text{C}-\text{O}-\text{CH}_2 \end{array} $	Diphosphatidylglycerol (cardiolipin)

(a)



1-Stearoyl-2-oleoyl-3-phosphatidylcholine

(b)

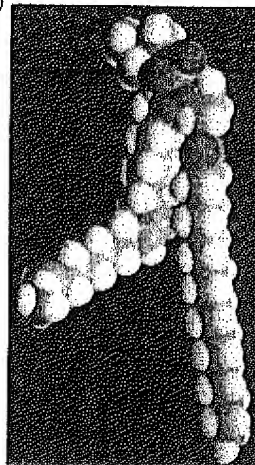
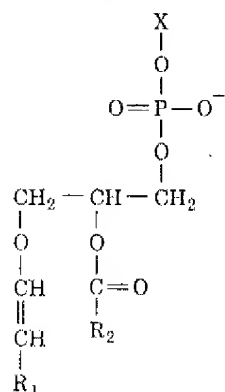


FIGURE 11-4. The glycerophospholipid 1-stearoyl-2-oleoyl-3-phosphatidylcholine: (a) molecular formula in Fischer projection and (b) space-filling model with H white, C gray, O red, and P green. [Courtesy of Richard Pastor, FDA, Washington, D.C.]

α,β -unsaturated ether linkage in the *cis* configuration rather than through an ester linkage.

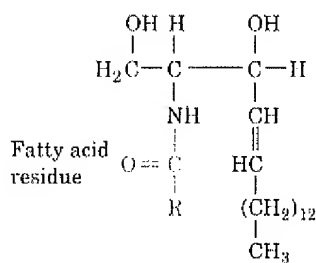


A plasmalogen

Ethanolamine, choline, and serine form the most common plasmalogen head groups.

D. Sphingolipids

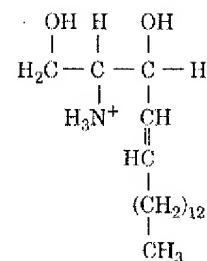
Sphingolipids, which are also major membrane components, are derivatives of the C_{18} amino alcohols **sphingosine**, **dihydrosphingosine** (Fig. 11-5), and their C_{16} , C_{17} , C_{19} , and C_{20} homologs. Their *N*-acyl fatty acid derivatives, **ceramides**,



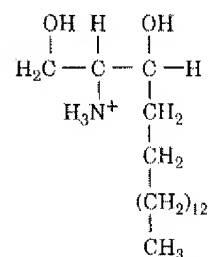
A ceramide

occur only in small amounts in plant and animal tissues but form the parent compounds of more abundant sphingolipids:

1. **Sphingomyelins**, the most common sphingolipids, are ceramides bearing either a phosphocholine (Fig. 11-6) or a phosphoethanolamine moiety so that they can also be classified as **sphingophospholipids**. Although sphingomyelins differ chemically from phosphatidylcholine and phosphatidylethanolamine, their conformations and charge distributions are quite similar. The membranous myelin sheath that surrounds and electrically insulates many nerve cell axons (Section 34-4C) is particularly rich in sphingomyelin.
2. **Cerebrosides**, the simplest **sphingoglycolipids** (alternatively **glycosphingolipids**), are ceramides with head groups that consist of a single sugar residue. **Galacto-**



Sphingosine



Dihydrosphingosine

FIGURE 11-5. The chiral centers at C2 and C3 of sphingosine and dihydrosphingosine have the configurations shown in Fischer projection. The double bond in sphingosine has the *trans* configuration.

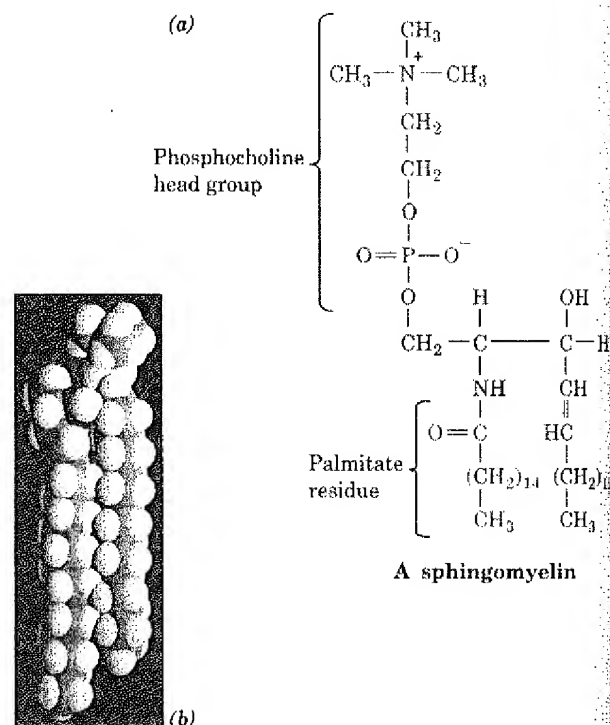
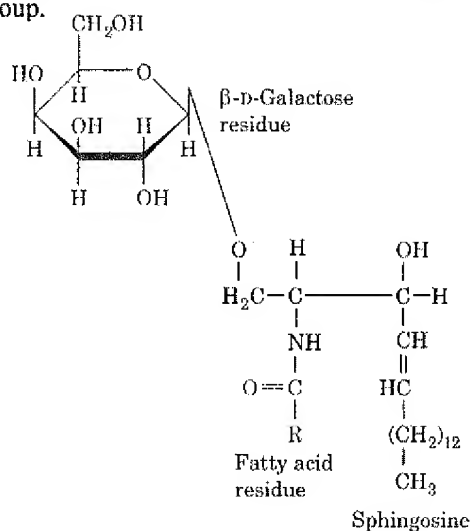


FIGURE 11-6. A sphingomyelin: (a) molecular formula in Fischer projection and (b) space-filling model with H white, C gray, N blue, and O red. Note its conformational resemblance to glycerophospholipids (Fig. 11-4). [Courtesy of Richard Pastor, FDA, Washington, D.C.]

cerebrosides, which are most prevalent in the neuronal cell membranes of the brain, have a β -D-galactose head group.



Glucocerebrosides, which instead have a β -D-glucose residue, occur in the membranes of other tissues. *Cerebrosides, in contrast to phospholipids, lack phosphate groups and hence are most frequently nonionic compounds.* The galactose residues of some galactocerebrosides, however, are sulfated at their C3 positions to form

ionic compounds known as **sulfatides**. More complex sphingoglycolipids have unbranched oligosaccharide head groups of up to four sugar residues.

3. **Gangliosides** form the most complex group of sphingoglycolipids. They are ceramide oligosaccharides that include among their sugar groups at least one sialic acid residue (*N*-acetylneuraminic acid and its derivatives; Section 10-1C). The structures of gangliosides G_{M1} , G_{M2} , and G_{M3} , three of the over 60 that are known, are shown in Fig. 11-7. Gangliosides are primarily components of cell surface membranes and constitute a significant fraction (6%) of brain lipids. Other tissues also contain gangliosides but in lesser amounts.

Gangliosides have considerable physiological and medical significance. Their complex carbohydrate head groups, which extend beyond the surfaces of cell membranes, act as specific receptors for certain pituitary glycoprotein hormones that regulate a number of important physiological functions (Section 34-4A). Gangliosides are also receptors for bacterial protein toxins such as cholera toxin (Section 34-4B). There is considerable evidence that gangliosides are specific determinants of cell-cell recognition, so they probably have an important role in the growth and differentiation of tissues as well as in carcinogenesis. Disorders of ganglioside breakdown are responsible for several heredi-

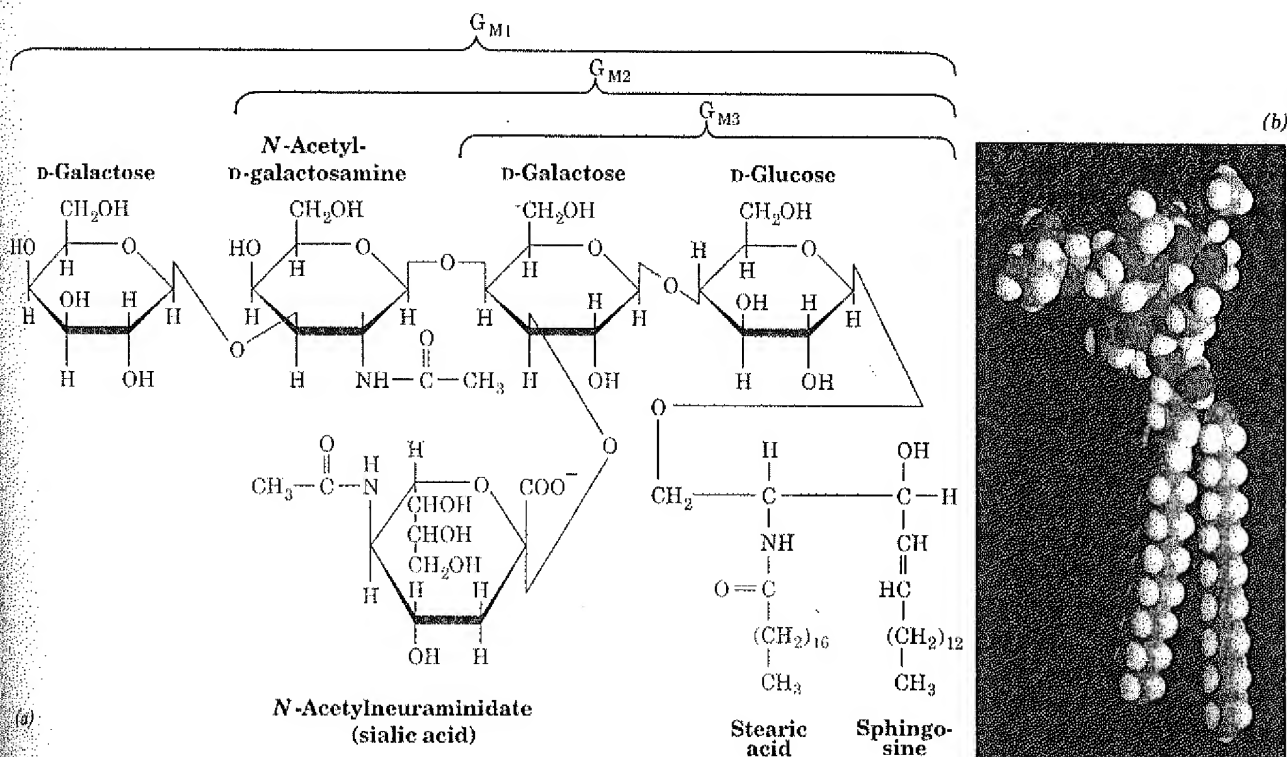


FIGURE 11-7. Ganglioside G_{M1} : (a) structural formula with its sphingosine residue in Fischer projection and (b) space-filling model with H white, C gray, N blue, and O red. Gangliosides G_{M2} and G_{M3} differ from G_{M1} only by the sequential absences of

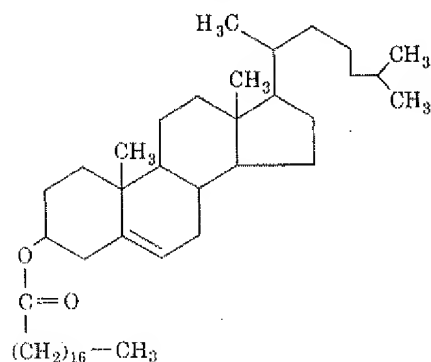
the terminal D-galactose and *N*-acetyl-D-galactosamine residues. Other gangliosides have different oligosaccharide head groups. [Courtesy of Richard Venable, FDA, Washington, D.C.]

tary **sphingolipid storage diseases**, such as Tay-Sachs disease, which are characterized by an invariably fatal neurological deterioration (Section 23-8C).

E. Cholesterol

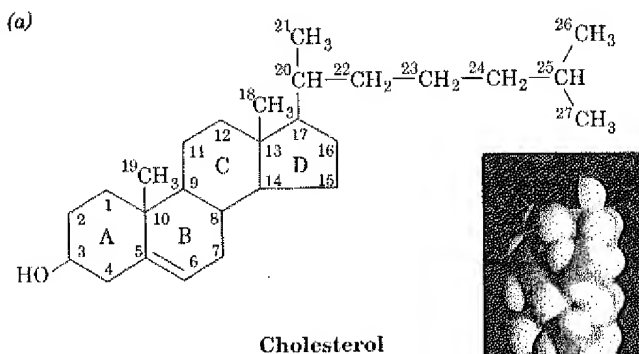
Steroids, which are mostly of eukaryotic origin, are derivatives of **cyclopentanoperhydrophenanthrene** (Fig. 11-8). The much maligned **cholesterol** (Fig. 11-9), the most abundant steroid in animals, is further classified as a **sterol** because of its C3 OH group and its branched aliphatic side chain of 8 to 10 carbon atoms at C17.

Cholesterol is a major component of animal plasma membranes and occurs in lesser amounts in the membranes of their subcellular organelles. Its polar OH group gives it a weak amphiphilic character, whereas its fused ring system provides it with greater rigidity than other membrane lipids. Cholesterol is therefore an important determinant of membrane properties. It is also abundant in blood plasma lipoproteins (Section 11-4), where ~70% of it is esterified to long-chain fatty acids to form **cholesteryl esters**.

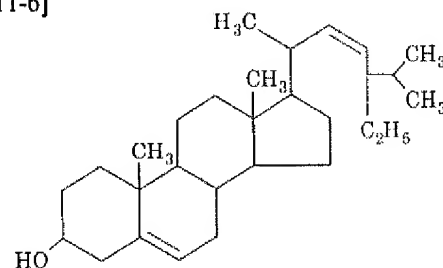


Cholesteryl stearate

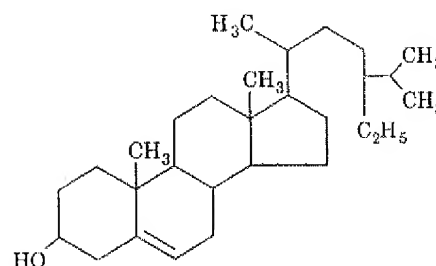
Cholesterol is the metabolic precursor of **steroid hormones**, substances that regulate a great variety of physiological functions including sexual development and carbohydrate metabolism (Section 34-4A). The much debated role of cholesterol in heart disease is examined in Section 11-4.



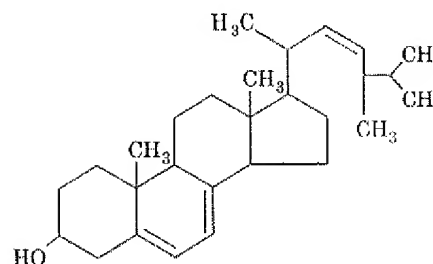
Plants contain little cholesterol. Rather, the most common sterol components of their membranes are **stigmasterol** and **β -sitosterol** [cut 11-6]



Stigmasterol

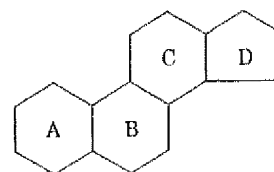


β -Sitosterol



Ergosterol

which differ from cholesterol only in their aliphatic side chains. Yeast and fungi have yet other membrane sterols such as **ergosterol**, which has a C7 to C8 double bond. Prokaryotes, with the exception of mycoplasmas (Section 1-1B), contain little, if any, sterol.



Cyclopentanoperhydrophenanthrene

FIGURE 11-8. Cyclopentanoperhydrophenanthrene, the parent compound of steroids, consists of four fused saturated rings. The standard ring labeling system is indicated.

FIGURE 11-9. Cholesterol: (a) structural formula with the standard numbering system and (b) space-filling model with H white, C gray, and O red. Cholesterol's rigid ring system makes it far less conformationally flexible than membrane lipids: Its cyclohexane rings can adopt either the boat or the chair conformations (Fig. 10-6) but the chair conformation is highly preferred. [Courtesy of Richard Pastor, FDA, Washington, D.C.]